MYCELIUM RADICIS ATROVIRENS IN FOREST SOILS, ISOLATION FROM SOIL MICROHABITATS AND IDENTIFICATION

WALTER GAMS

Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Getreide-, Oelfrucht- und Futterpflanzenkrankheiten, Kiel-Kitzeberg

Introduction

In recent years, new isolation techniques have been developed for ecological investigations of soil fungi, which facilitate more frequent isolation of sterile fungi (PARKINSON and WILLIAMS, 1961; GAMS, 1959; GAMS and PARKINSON, 1961). Dark sterile mycelia have frequently been isolated from forest soils.

One type of such mycelia is known for a long time as *Mycelium radicis atrovirens* MELIN from the mycorrhiza literature, after it was isolated from conifer roots and described by MELIN (1923). Since then, it was repeatedly isolated from roots of various forest trees (cf. MAŃKA, 1960). A closely related or possibly identical fungus was isolated by LAGERBERG, LUNDBERG and MELIN (1928) from decaying wood and therefore called *Zylomyces*.

Methods of Isolation

In mycorrhiza investigations fungi are currently isolated from washed or surface sterilized ($HgCl_2$ 0.1% for 30 sec) roots. This is probably the reason why *M.r.atrovirens* was observed for the first time on roots, since the isolation of sterile mycelia becomes feasible to a larger extent by the use of washing techniques. Washing of other soil constituents has become fashionable only in the last years. *M.r.atrovirens* remained therefore unknown in other soil fungal investigations when mainly spore isolation techniques were used.

In our experiments *M.r.atrovirens* was isolated successfully with the washing apparatus developed by PARKINSON and WILLIAMS (1961) and with

the same success by cellophane strips from which sterile celllolytic fungi can be isolated (GAMS, 1960). The strips had to be buried in the soil for at least two weeks. The polythene strip technique (GAMS, 1959) was found less suitable, because it is more difficult to remove spores without loosing mycelium. Finally, in an investigation of fir mycorrhiza, the original washing and surface sterilizing technique by MELIN (1923) was used, the latter being more successful because it suppresses bacteria and other fungi.

Investigations on the distribution of *M. r. atrovirens*

Our observations involve experiments in three different regions: (1) Delamere forest podzol, Chesh. (work done in 1960/61 at The Hartley Botanical Laboratories, Liverpool) (cf. BURGES, 1962), (2) various forest soils in Tyrol: A brown rendzina near Imst (ca. 800 meters altitude, from pine roots), acidic humus under spruce forest, covered with *Vaccinium vitis idaea* and a *Sphagnum* peat with *Pinus montana* at Pillersattel (ca. 1400 meters, isolations from cellophane), (3) mycorrhiza of fir *(Abies alba)* in the French Jura (4 sites between 450 and 750 meters alt. and in the Savoyan Prealps near Larringes (800 meters), isolations from roots and cellophane.

The most extensive work was done at Delamere forest, where various isolation techniques have been tried for the isolation of actively growing fungi. With regard to *M.r.atrovirens*, the following results were obtained: Warcup's soil plates —, Polythene strips +, Cellophane strips ++, washed soil particles +. It was not isolated very frequently from washed particles because of the high activity of fast growing species which tended to overgrow it. The following figures (experiment from 23 July 1961) illustrate the distribution on different kinds of particles (expressed as percentage of plated particles):
amorphous 0, pieces of bark 1.4, wood fragments 0, roots 4.0 %. The absolute figures are very small, subjected to strong variation. Seasonal fluctuations are illustrated by the following figures:

	10 Nov.	8 March	18 Apr.	3 May	23 July
% *M.r.atrov.*	4,3	0,8	0	0	1.4
CR	1.3	1.5	2.2	2.3	2.3

These figures could suggest a decline during spring and summer. We are however rather inclined to think of a general increase of fungal activity in these months, so that slow growing species are overgrown by spreading species. This can be seen by a comparison of the CR values (colonization rate = colonies per plated particle) in the above table.

M.r.atrovirens was not only isolated by the author in the H-layer, but also by KENDRICK (1958) in the upper and by WILLIAMS (1962) in the lower horizons. It shows an exception from the rule of strict vertical localization of species in this soil profile. WILLIAMS observed this fungus also on dilution plates poured from the washing water of the washing apparatus.

During the above mentioned work in France, *M.r.atrovirens*-like fungi were regularly observed on fir roots and on cellophane, however the fungus could be isolated only very rarely from the Jura stations (on 1% or less of the plated fragments) because it was frequently overgrown by fast growing species; at the sampling site near Larringes it was dominating with a frequency of ca. 25% of the plated root fragments and also on cellophane.

Identification

MELIN (1923) gives a very careful macroscopical and microscopical description of *M.r.atrovirens*. We can complete it by the description of the behaviour on cellophane: it forms rather coarse rooting branches (TRIBE 1960) of olivaceous colour, which show a moderate orientation in the direction of the cellulose fibrils, the single branches are separated from each other. They darken with age at the centre and form sclerotium-like structures. The fungus is cellulolytic, as it was shown already by MELIN (1925) and LEVISOHN (1954). The adverse observations by SCHELLING (1952) may be due to the fact that she used neither trace elements nor growth factors in her experiments. Rooting branches of the *M.r.atrovirens* type were also observed on chitin strips (GRAY, 1962) in soil from spruce forest at Pillersattel, but it could not be isolated because it was overgrown by mucoraceous fungi.

Nevertheless, as a sterile mycelium, *M.r.atrovirens* does not show sufficent morphological differentiation, to secure a definite identification. The only exact method remains therefore the comparison with type strains and the experiment of anastomosing between different strains (BULLER, 1933).

Two strains from the Centraalbureau voor Schimmelcultures at Baarn, which were isolated by LEVISOHN from *Pinus silvestris* and *Picea sitchensis* respectively, differed from our strains and from Melin's description by much narrower hyphae. By courtesy of Prof. E.Björkman, we obtained an original strain isolated by Melin which showed much less aerial mycelium than our strains, possibly as a result of degeneration, but otherwise agreed perfectly. Our strains from different sources agreed with each other and the description given by Melin.

We observed anastomoses between several of our strains from different origins. Anastomoses with Melin's strain were formed only incompletely by some strains from Delamere and some alpine isolates. From these observations the Delamere strains can be divided into two or more groups which do not anastomose with each other.

Sporulation

A few strains from Delamere could be induced to sporulate: On CMC-Agar (GAMS, 1960) conidia of *Phialocephala dimorphospora* KENDRICK (1961) were formed after two or three months at low temperatures (2 and 10°C). Another strain of the same conidial type, isolated from peat at Wildmoos near Seefeld-Tyrol, sporulated after a short time at room temperature on malt extract agar. The two sporulating types did not anastomose, neither did the sporulating strains from Delamere anastomose with the completely sterile ones.

Therefore we consider *M.r.atrovirens* to be genetically heterogenous, which however can only be proved by delicate methods. The investigations are not yet finished. The author would appreciate to obtain isolates of similar type from other workers.

Conclusion

As Prof. BJÖRKMAN (personal communication) informed us, *M.r.atrovirens* is a true soil fungus of wide distribution, able to grow saprophytically and independently from roots. The rare isolation by Williams from dilution plates indicates that it sporulates occasionally also in the soil. Much further work on its ecology is needed. We know far too little about its possible

importance as a root parasite of conifers (LINDQUIST, 1937 and 1939) or its interaction with mycorrhiza formation.

An interesting result is the similarity of results obtained with washing techniques and cellophane strips. By both ways sterile soil fungi were frequently isolated.

The observation of sporulation is a further proof of the suitability of cellulose-containing agar in inducing sporulation, as it was shown by GAMS (1960) for Carboxymethylcellulose and by SLOAN, ROUTIEN and MILLER (1960) for Alphacel.

Acknowledgments

The author gratefully acknowledges fruitful collaboration with the soil microbiologists from Liverpool Hartley Botanical Laboratories. He is also greatly endebted to Prof. Björkman for supplying a type strain and helpful suggestions.

Summary

Sterile dark mycelia of *Mycelium radicis atrovirens* type were commonly isolated from forest soils of very different type, from washed roots and other soil particles, and from cellophane strips. A few strains developed conidia of *Phialocephala dimorphospora* KENDRICK.

References

BULLER, A. R. H., 1933. Researches on fungi. Vol. *V*, London.

BURGES, N. A., 1962. Contribution to this Symposium.

GAMS, W., 1959. Isolierung von Hyphen aus dem Boden. Sydowia, **13**, 87—94.

———, 1960. Studium zellulolytischer Bodenpilze mit Hilfe der Zellophanstreifen-Methode und mit Carboxymethyl-Zellulose. Sydowia, **14**, 295—307.

——— and D. PARKINSON, 1961. Problematik der Bodenmykologie. Ber. Geobot. Inst. ETH, Stiftung Rübel, **32**, 176—186.

GRAY, T. R. G., 1962. Contribution to this symposium.

KENDRICK, W. B., 1958. Microfungi in Pine litter. Thesis Liverpool.

———, 1961. The *Leptographium* Complex, *Phialocephala* gen. nov. Can. J. Botany, **39**, 1079—1085.

LAGERBERG, T., G. LUNDBERG and E. MELIN, 1928. Biological and practical researches into blueing in pine and spruce. Svenska Skogsv. Fören. Tidsk., **25**, 145—272.

LEVISOHN, I., 1954. Test for the pseudomycorrhizal group of soil fungi. Nature, Lond., **174**, 408—409.

LINDQUIST, B., 1937. Om några parasitiska marksvampar i nordsvenska råhumusmarker. Norrlands Skogsvårdsförbunds Tidskrift, Heft **2**.

———, 1939. Die Fichtenmykorrhiza im Lichte der modernen Wuchsstofforschung. Bot. Notiser, **1939**, 315—356.
MAŃKA, K., 1960. O grzybie korzeniowym *Mycelium radicis atrovirens* MELIN (Der Wurzelpilz M.r.a. Melin). Monographiae Botanicae, **10**, 147—158.
MELIN, E., 1923. Experimentelle Untersuchungen über die Konstitution und Oekologie der Mykorrhizen von *Pinus silvestris* und *Picea Abies*. Mykolog. Untersuchungen u. Berichte von R. Falck, **2**. Cassel.
———, 1925. Untersuchungen über die Bedeutung der Baummykorrhiza, eine ökologisch-physiologische Studie. Jena.
PARKINSON, D. and S. T. WILLIAMS, 1961. A method for isolating fungi from soil microhabitats. Plant and Soil, **13**, 347—355.
SCHELLING, C. L., 1952. Zur Kenntnis der Physiologie von *Mycelium radicis atrovirens* Melin mit besonderer Berücksichtigung der Verwertbarkeit verschiedener Kohlenstoffquellen. Diss. ETH, Zürich.
SLOAN, R. J., J. B. ROUTIEN and V. P. MILLER, 1960. Increased sporulation in fungi. Mycologia, **52**, 47—63.
TRIBE, H. T., 1960. Microbiology of cellulose decomposition in soil. Soil. Sci. **92**, 61—77.
WILLIAMS, S. T., 1962. Contribution to this symposium.

Discussion

W. GAMS: Since the paper was written, I have examined numerous strains from the origins mentioned in the paper and from others with the methods described, but sporulation was never observed again. Even in the soil, where it was observed first, in Delamere forest, it does not seem to be a uniform fungus species, and therefore it appears to be wiser to speak of *M.r.atrovirens* and not of *Phialophora* throughout. (cf. Williams' contribution to this symposium).
G. MINDERMAN: Is there any connection between *M.r. atrovirens* and the old copper wire, which may be connected with *Cenococcum* or *Humicola.*
W. GAMS: From these two genera *M.r. Atrovirens* can clearly be distinguished. *Humicola* has hyaline mycelium and dark chlamydospores, whereas the *M.r.a.* mycelium is more or less dark throughout and has no differentiated chlamydospores. *Cenococcum* shows darker cultures, no greenish hue and it grows much slower. Its hyphae are very fragile. Ecologically they are well distinct: *Cenococcum* is a true mycorrhizal fungus restricted in its microhabitat, whereas *M.r.a.* grows profusely on the root surfaces and penetrates into the superficial layers of cortex cells, it grows also saprophytically through the soil.
A. BURGES: Dr. Gams' paper illustrates a very difficult problem in soil microbiology. Many of the hyphae we see cannot be identified yet they are very important quantitatively. The identification of even one species may require a great deal of work.
D. PARKINSON: I would like to mention the fact that *Phialophora* (*M.r.a.* according to Dr. Gams) is isolated frequently from dead roots of *Pinus* and *Pteridium* at Delamere, and from the internal tissues of living (old) roots growing in this soil. Presumably it is at this stage, playing a part in the decomposition of the plant tissues.
H. T. TRIBE: Does *M.r.a.* occur on plant roots in arable soils? Dark sterile mycelia are quite common on older plant roots in these soils.
W. GAMS: No, they are different.
A. E. APINIS: *M.r.a.* was not recorded from soils of coastal grasslands mentioned in my paper. Apparently this fungus is confined to certain biocoenoses, such as pine forest of Delamere in Cheshire, England.
D. PARKINSON: Sterile dark fungi are reported as colonizers of living roots in a wide

variety of soils. Although these organisms appear in low frequencies in soil, and are slow growing in pure culture, they seem to be rapid colonizers of young developing roots.
S. T. WILLIAMS: Which character does Dr. Gams use to define *M.r.a.*
W. GAMS: It is defined firstly by its habitat on tree roots and secondly by comparison of its morphology in culture with type strains.
T. F. HERING: Has there been any study of mycelial anastomosis between strains.
W. GAMS: The strains are heterogenous in nature and do not anastomose.
G. W. HARMSEN: Sterile mycelium is quantitavely important but what about metabolic activity?
W. GAMS: The only information I have is the observation of cellophane decomposition at Delamere forest, *M.r.a.* is certainly the most important organism in this process, but its activity is still low as compared with that of other fungi in other soils.
D. PARKINSON: Slow growth does not necessarily mean low metabolic activity.